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## Impact of a microphallid trematode on the behaviour and survival of its isopod intermediate host: phylogenetic inheritance?

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**Abstract** The extent to which the ability of parasites to alter host behaviour is phylogenetically inherited as opposed to independently evolved has received little attention. We investigated the impact of an undescribed species of *Microphallus* on the behaviour and survival of its host, the freshwater isopod *Austridotea annectens*, to determine if it produced effects comparable to those induced by other trematodes of this genus. There was no difference between the vertical distribution and responses to light of infected isopods and those of uninfected isopods. In contrast, we found that infected isopods were more active swimmers than uninfected isopods, and that they failed to show the evasive responses shown by uninfected isopods when exposed to a simulated predator. There was no detectable effect of *Microphallus* infection on isopod survival, however. Overall, our results provide no strong evidence that the behavioural manipulation shown by our *Microphallus* species and that displayed by the well-studied *Microphallus papillorobustus* are inherited from a common ancestor rather than independently derived.

acanthocephalans, modification of host behaviour appears to be an ancestral trait now shown by most if not all extant species (Moore 1984), in other groups only certain genera or species are capable of changing host behaviour. Several authors (e.g. Moore and Gotelli 1990, 1996; Poulin 1995, 1998) have argued that we could better understand the evolution of host behaviour modification by parasites, whether it is adaptive or not, by placing it within a comparative or phylogenetic context.

Among trematodes, the ability to modify host behaviour is widespread though not universal (Moore 2002). For instance, consider the trematode genus *Microphallus* (family Microphallidae). In most species of this genus, adult worms live in birds; snails are used as first intermediate hosts and crustaceans as second intermediate hosts. In theory, metacercariae in crustaceans could benefit by altering the behaviour of their host in ways that increase its susceptibility to predation by avian definitive hosts. Indeed, metacercariae of *M. papillorobustus* induce profound behavioural changes in their amphipod intermediate hosts, causing them to swim at the water surface instead of at the bottom of the water column like uninfected amphipods (Helluy 1983). This marked behavioural alteration not only makes infected amphipods more prone to bird predation, it also impacts other aspects of their biology such as the type of mating partners they can obtain (Thomas et al. 1996). This parasite is now used as a textbook example of a parasite capable of manipulating host behaviour (e.g., Combes 2001). Metacercariae of *M. papillorobustus* can also induce mortality in their amphipod host (Thomas et al. 1995); in other words, they can kill their host directly in an intensity-dependent way, in addition to making them more vulnerable to bird predation. This type of virulence may be common in microphallids, as it has been reported in other genera (e.g., *Maritrema*: see Meissner and Bick 1999; Fredensborg et al. 2004). The real question is whether host behavioural alteration is an ability common to all species of the genus *Microphallus*, possibly inherited from their common ancestor, or whether it is only observed in certain species and not others.

### Introduction

Alterations in host behaviour induced by parasites have been reported across all major host and parasite phyla, suggesting that they have evolved independently numerous times (Moore 2002). There are, however, phylogenetic influences acting on the ability of parasites to alter host behaviour. Whereas in some groups, such as

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Only two other *Microphallus* species have been investigated with respect to changes in host behaviour induced by metacercariae. In the first, *Microphallus turgidus*, changes in the behaviour of the shrimp second intermediate host were observed, but they were less pronounced and more likely to reflect pathological effects than some adaptive manipulation by the parasite. Infected shrimp had lower swimming stamina than uninfected shrimp, and spent less time motionless in the presence of a fish predator (Kunz and Pung 2004). In the absence of predators, the behaviour of infected shrimp was roughly identical to that of uninfected shrimp (Khan et al. 2003). Also, infection by *M. turgidus* does not appear to affect shrimp survival (Pung et al. 2002). In the second case, an undescribed New Zealand *Microphallus* species, the situation is slightly different because this species has an abbreviated life cycle: the metacercariae encyst in the snail first intermediate host, so that the snail serves as both first and second intermediate hosts. Snail behaviour is modified significantly: infected snails respond differently to gravity and spend more time attached to the top of rocks than uninfected snails (Levri 1999; Levri and Fisher 2000). In the two species with the typical three-host life cycle, we therefore have one, *M. papillorobustus*, capable of clear-cut and clearly adaptive host behaviour manipulation, and the other, *M. turgidus*, inducing weaker changes more likely to be pathological side effects.

Here, we investigate a new *Microphallus* species from New Zealand. It differs from the one studied by Levri (1999) and Levri and Fisher (2000) in that it displays the typical three-host cycle. The snail *Potamopyrgus antipodarum* serves as first intermediate host, in which the parasite multiplies asexually. Cercariae leaving the snail then encyst as metacercariae in the freshwater isopod *Austrodeonta annectens*. We examine the influence of the parasite on both isopod behaviour and isopod survival. In our study of isopod behaviour, we looked at both the spontaneous behaviour of uninfected and infected individuals, as well as their responses to a simulated predator. Our results shed new light on whether or not the ability to manipulate the crustacean second intermediate host is a generic trait of the genus *Microphallus*, which is now a textbook case study.

## Materials and methods

### Isopod collections and handling

Isopods were collected from Lake Waihola, located 40 km southwest of Dunedin, South Island, New Zealand. They were captured by dragging a dipnet through beds of macrophytes, 2–5 m from the shore. In the laboratory, isopods were kept in small groups in 4 L containers filled with aerated lake water, at  $20 \pm 2^\circ\text{C}$ , and fed with macrophytes and detritus from the lake. Each isopod was used in only one of the following experiments, and all were used within one week of capture. Infection status

was unknown until each isopod was dissected following an experiment, and thus all tests were performed blind.

After each of the experiments described below, each isopod was sexed (if reproductively mature), measured (length from tip of cephalon to end of uropod), and dissected to determine infection status. The number of *Microphallus* metacercariae in each isopod was recorded, as well as their exact location in the isopod's body, which ranges from the tissue within the pleopods to the cephalon. Since a preliminary analysis could find no influence of the location of the parasites on the behaviour of their host, we only present results pertaining to the number of parasites per host. These data (as well as the behavioural scores described below) did not meet the assumptions of normality, and thus non-parametric statistical tests were used.

### Spontaneous behaviour of isopods

In the first experiment, the vertical distribution and swimming activity of undisturbed isopods were quantified. Individual isopods ( $N=150$ ) were placed in clear plastic cylinders (6-cm high and 1.5-cm diameter) filled with aerated lake water. Each cylinder was divided into six depth sections, each 1-cm high. To eliminate any phototropism, a broad and opaque covering plate was placed on the cylinders, such that uniform lighting came only from the sides of the cylinders. After a 5 min acclimation period, the vertical position of each isopod was recorded ten times, at 30 s intervals; position was scored each time ranging from 1 (bottom of the cylinder) to 6 (top of the water column). At each of the ten recording times, the swimming activity of each isopod was also recorded, as either 0 (making no swimming movement) or 1 (actively swimming). Scores of each individual were averaged across all ten observations, for both vertical position and swimming activity.

The second experiment measured the reaction to light, or phototropism, of undisturbed isopods. Individual isopods ( $N=150$ ) were placed in a covered petri dish (4.5-cm diameter), half of which was fully covered in dark opaque material. Each dish was filled with aerated lake water and placed 2 m below a 60 W fluorescent tube. In each trial, an isopod was introduced in the middle of the dish and allowed 5 min to acclimate. Its position was then recorded ten times, at 30 s intervals, as either 0 (under the opaque half of the cover) or 1 (in the clear part of the dish). The scores of each individual were summed across all ten recording times, and thus could range from 0 (strongly photophobic) to 10 (strongly photophilic); a score of five would indicate no preference with respect to light.

### Isopod response to a simulated predator

The response of individual isopods ( $N=43$ ) to a model predator was estimated by comparing their use of refuge

before and after exposure to the predator stimulus. The experimental container (25 × 25 cm) was filled with 4 L of aerated lake water; its bottom was haphazardly covered with 45 leaf disks (2-cm diameter), made of partially decomposed leaves collected from the lakeshore. The discs provided both food and shelter for the isopods. After an isopod was placed in the centre of the container and given 5 min to acclimate, its position was recorded ten times at 30 s intervals, as either fully under shelter (1), partially sheltered and thus partially visible (2), or fully exposed (3). After the tenth recording, a model duck was used to present a predatory stimulus. The duck's bill was used to "search" the bottom of the container for 20 s in the manner of a dabbling duck. Following this stimulus, the position of the isopod was again recorded ten times at 30 s intervals, as before. The scores of each individual were averaged across all ten recordings, separately for both before and after exposure to the predator stimulus.

### Isopod survival

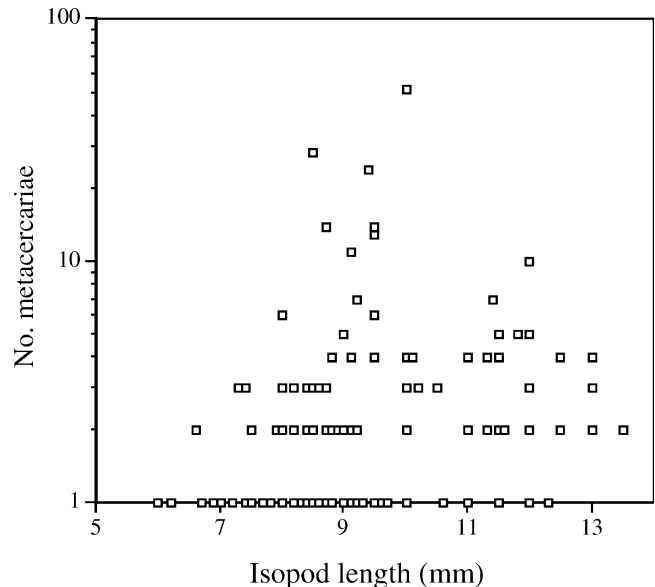
Individual isopods ( $N=35$ ) were placed in separate containers (6-cm high and 3-cm diameter), each filled with aerated lake water. All containers were checked four times a day, to assess how long the isopods survived under those conditions to the nearest 6 h.

## Results

Of the 343 isopods used in behavioural studies, 58% were parasitized by *Microphallus*, harbouring between 1 and 51 metacercariae (the vast majority harboured fewer than five metacercariae). Among the individuals that could be sexed, there were more females than males (241 vs. 90). Males harboured slightly more metacercariae than females on average (Mann-Whitney  $U$ -test,  $Z=4.95$ ,  $N=331$ ,  $P<0.001$ ). However, they are also on average 2 mm longer than females ( $Z=11.88$ ,  $N=331$ ,  $P<0.0001$ ), and since isopod length correlates positively with the number of metacercariae harboured (Spearman rank correlation:  $r_s=0.410$ ,  $N=343$ ,  $P<0.0001$ ), it is most likely that host size is the main determinant of infection intensity. The relationship between isopod length and infection intensity is not particularly strong, however, and shows much scatter (Fig. 1).

### Spontaneous behaviour of isopods

There was no evidence that *Microphallus* infection affected the vertical position of isopods. No difference was found between the vertical position scores of infected and uninfected isopods (Mann-Whitney  $U$ -test:  $Z=0.86$ ,  $N=150$ ,  $P=0.40$ ), and there was no relationship between intensity of infection and vertical position score among the infected isopods (Spearman rank



**Fig. 1** Relationship between body length and the number of *Microphallus* metacercariae per host in the isopod *A. annectens*. The figure includes only the 200 isopods that were parasitised out of the 343 examined; many of these harboured a single metacercaria, and thus many points are stacked along the  $x$ -axis. Note the logarithmic scale on the  $y$ -axis

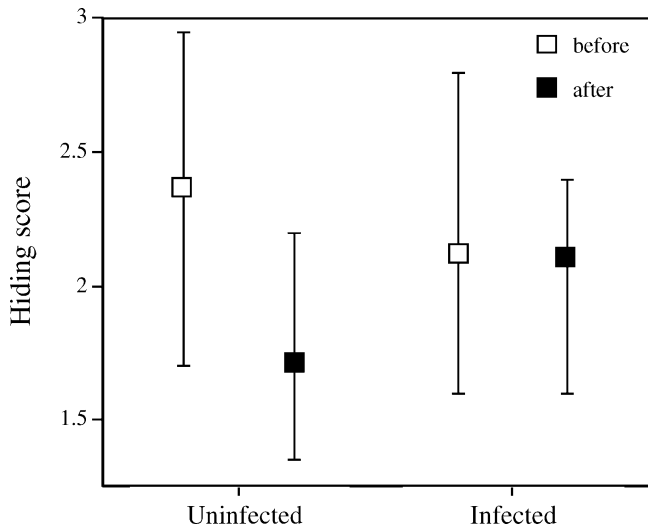
correlation:  $r_s=0.010$ ,  $N=91$ ,  $P=0.93$ ). As a rule, all isopods spent more time in the bottom two zones of the cylinders.

In contrast, isopods parasitized by *Microphallus* metacercariae were significantly more active than those that were uninfected ( $Z=8.05$ ,  $N=150$ ,  $P<0.001$ ). Based on their activity scores, on average infected isopods were seen actively swimming 45% of the time as opposed to close to 0% for uninfected isopods. There was also a positive correlation between infection intensity and the activity score of infected isopods ( $r_s=0.604$ ,  $N=91$ ,  $P<0.0001$ ).

In the experiment measuring the isopods' reaction to light, most individuals showed mild to pronounced photophobia (most scores  $< 5$ ). There was no difference, however, between the scores of infected and uninfected isopods ( $Z=0.99$ ,  $N=150$ ,  $P=0.32$ ), and among infected isopods there was no correlation between infection intensity and the light reaction score ( $r_s=-0.103$ ,  $N=82$ ,  $P=0.35$ ).

### Isopod response to a simulated predator

Following exposure to a model predator, uninfected isopods showed a significantly lower hiding score than they did before (paired  $t$ -test, two-tailed:  $t=2.29$ , d.f. = 15,  $P=0.037$ ), indicating that they spent more time under the cover of leaves after the stimulus (Fig. 2). In contrast, infected isopods had the same score before and after exposure to the model predator ( $t=1.27$ , d.f. = 26,  $P=0.238$ ), indicating that they did not respond to the



**Fig. 2** Median (and interquartile range) hiding score of 16 uninfected isopods and 27 isopods infected by metacercariae of the trematode *Microphallus*, both before and after exposure to a simulated predator

appearance of the predator threat. By subtracting the score obtained before from that obtained after, we could measure the magnitude of any response; among infected isopods, this response did not correlate with intensity of infection ( $r_s = 0.043$ ,  $N = 27$ ,  $P = 0.83$ ).

#### Isopod survival

Isopod survival varied widely among individuals, ranging from less than 90 to more than 1000 h. Uninfected isopods did not survive longer than isopods harbouring *Microphallus* metacercariae ( $Z = 0.22$ ,  $N = 35$ ,  $P = 0.83$ ). Among infected isopods only, there was no relationship between intensity of infection and how long the host survived ( $r_s = -0.023$ ,  $N = 16$ ,  $P = 0.38$ ). There was also no correlation between isopod survival and isopod body length, among either infected or uninfected isopods (both  $P > 0.23$ ).

#### Discussion

The degree to which the ability to modify host behaviour is inherited as opposed to having evolved independently remains unresolved for most parasite taxa known to alter the behaviour of their host. Here, we investigated a congeneric species of *Microphallus papillorobustus*, one of the best-documented manipulative trematodes (Helluy 1983; Thomas et al. 1995, 1996). Our results suggest that there are both similarities and differences between what these *Microphallus* parasites can do to their second intermediate hosts.

The *Microphallus* species we studied apparently induces its isopod host to be more active, i.e. spend more time swimming, and it also suppresses some of the

isopod's responses to the immediate threat of predation. Increased activity following infection is not uncommon in crustaceans parasitized by larval helminths (Poulin et al. 1992; Urdal et al. 1995; see Moore 2002). Similarly, altered responses to predators are also often associated with parasitic infection (Hechtel et al. 1993; see review in Moore 2002). Of course, our results were obtained from naturally infected hosts, and not via experimental infection; thus, it remains possible that these features of host behaviour are associated with infection without being caused by it. Still, assuming that infection does induce these behavioural differences, they have one feature in common with those produced by *M. papillorobustus*: they appear well capable of increasing the parasite's transmission to definitive hosts. Apart from ducks, the only other predator of isopods are fish (the common bully, *Gobiomorphus cotidianus*). These fish, however, can only feed on the smallest isopods, which are generally uninfected. Therefore, both our *Microphallus* and *M. papillorobustus* induce behavioural changes in their crustacean hosts that can facilitate the parasite's passage to bird hosts.

There are, however, important differences between what these parasites do to their hosts. First, whereas *M. papillorobustus* causes its host to swim at the water surface (Helluy 1983), the *Microphallus* species investigated here induces neither photophilia nor a tendency to swim higher in the water column independent of light. Second, whereas a single *M. papillorobustus* in the right location is sufficient to induce a full change in behaviour (Helluy 1983), in our study behavioural changes were intensity-dependent: the more metacercariae in a host, the more pronounced the change in behaviour. Third, whereas the main behavioural alteration induced by *M. papillorobustus* is independent of the immediate presence of a predator, one of the behavioural changes we found in the present study is only apparent in response to a predator. Fourth, whereas *M. papillorobustus* induces intensity-dependent mortality in its crustacean host (Thomas et al. 1995), our species did not influence the survival of its host under constant laboratory conditions but without food.

The first difference above is telling. Recently, it has been found that the manipulative *M. papillorobustus* affects the neurochemistry and neuronal architecture of the brain of its amphipod host (Helluy and Thomas 2003). This is achieved by the metacercariae that encyst in the cerebral region of amphipods; the others have no influence on host behaviour. If this neurobiological takeover of the host was a generic trait of *Microphallus*, resulting in altered sensory perception in the host with respect to light or gravity, we might expect it to occur in other species. In our species, we found no evidence for an equivalent behavioural change. In addition, metacercariae in the head region of isopods have no more effect on their behaviour than other metacercariae (unpublished results). Thus, although changes in host behaviour are associated with both *Microphallus* species, their proximate origins are not necessarily the same.



Two of the other differences, i.e. behavioural changes are intensity-dependent and there is no apparent parasite-induced mortality in our species, may have something to do with the identity of the crustacean host. *M. papillorobustus* uses a gammarid amphipod as its second intermediate host (Helluy 1983). Our *Microphallus* species uses an isopod, while *M. turgidus* uses a decapod, i.e. a shrimp (Pung et al. 2002). Not only do the latter two hosts belong to different crustacean orders, they are also much larger than amphipods. The size of *Microphallus* metacercariae does not vary substantially among species within the genus, and therefore those in isopods or shrimps are smaller relative to their hosts than the ones in amphipods. This may place a limit on the deleterious effects that the parasites may have, as well as constraining the magnitude of the behavioural change that a single metacercaria can achieve. Note that in some situations isopods can have their behaviour altered by a single parasite larva, i.e. a single acanthocephalan cystacanth can cause substantial change in its isopod host (Hechtel et al. 1993; Moore 1983; see other examples in Moore 2002). Acanthocephalan cystacanths, however, are much larger than microphallid metacercariae. In any event, the relatively larger size of the second intermediate host may explain why our findings are closer to those from studies on *M. turgidus* (Pung et al. 2002; Khan et al. 2003; Kunz and Pung 2004), which also exploits a large crustacean, than those relating to *M. papillorobustus*. Both *M. turgidus* and our species induce modest behavioural changes and little, if any, mortality in their crustacean host.

In conclusion, the *Microphallus* species we investigated seems capable of altering the behaviour of its isopod host in ways that may improve its transmission success to bird hosts. However, the nature of the behavioural changes it induces, and its undetectable effect on host survival, differ from those observed in the well-studied *M. papillorobustus*-amphipod system. A comparison of the proximate mechanisms used by these two species to effect the behavioural change in the host may be instructive. At this point, though, there is no strong evidence that the behavioural manipulation shown by our *Microphallus* species and that displayed by *M. papillorobustus* are inherited from a common ancestor rather than independently derived.

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